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Award Number: W81XWH-10-1-0400

TITLE: Glyco-Immune Diagnostic Signatures and Therapeutic Targets of Mesothelioma

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REPORT DATE: July 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
1 July 2011	Annual	06/15/2010-06/14/2011

4. TITLE AND SUBTITLE: Glyco-Immune Diagnostic Signatures and Therapeutic Targets of Mesothelioma

5a. CONTRACT NUMBER
W81XWH-10-1-0400

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES):
New York University School of MedicineNew York, NY 10016

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES):
U.S. Army Medical and Material Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This focus of this grant is to investigate immunoprofiles for serum antibodies to aberrant glycans in human and animal models of mesothelioma. This is accomplished using a one of a kind printed glycan array which is at NYU School of Medicine (NYUSoM). It is hoped that these experiments will allow us to diagnose and prognosticate mesothelioma more accurately in the future. We have been severely limited by our ability to start the human mesothelioma glycoprofiles as well as the animal profiles due to delivery and set up times for our one of a kind glycomics laboratory at NYUSoM. We summarize the situation in the progress report with the good news that we will be moving onwards in June with these studies.

15. SUBJECT TERMS
Malignant Mesothelioma; Glycan Array; Immunoprofiles; Robotic Arrayer

16. SECURITY CLASSIFICATION OF: U	**17. LIMITATION OF ABSTRACT: UU**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRCC

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INTRODUCTION

This project is funded in order to investigate immunoprofiles for serum antibodies to aberrant glycans in human and animal models of mesothelioma. This is accomplished using a one of a king printed glycan array which us at the New York University School of Medicine (NYUSoM). It is hoped that these experiments will allow us to diagnose and prognosticate mesothelioma more accurately in the future, and this has implications for the military since a number of individuals are exposed to the carcinogen agent, asbestos in the military.

BODY

We have been severely limited by our ability to start the human mesothelioma glycoprofiles as well as the animal profiles due to delivery and set up times for our one of a kind glyco laboratory at NYUSoM. We summarize the situation below with the good news that we will be moving onwards in June with the mesothelioma studies.

Pushback of MicroGrid Delivery Date

After a Microgrid II robotic arrayer was purchased from Digilab in the spring of 2010, an estimated delivery date was set for August 2010. However, because of manufacturing issues, Digilab pushed back their estimate to late September. After speaking with the company about three additional missed deadlines the Digilab COO made a placeholder unit available. The unit arrived in early November and was made operable by Digilab engineers soon after arrival. A series of Optimization steps had to follow.

Optimization of Slide Hybridization & Quantification

During the period before a functional arrayer was available, optimization of techniques for slide hybridization and analysis using slides that had already been printed at the Scripps Research Institute was the primary focus. Technicians were trained in slide hybridization and development by Dr. Margaret Huflejt. From August to October attempts were made to improve slide quality by reducing background florescence and increasing consistency between replicates. However Scripps printing protocols which are not tailored for high consistency between prints and print batches limited technical achievements. Technical replicates of slide quantification proved not to be reproducible. Hence we moved onto optimization in our own laboratory.

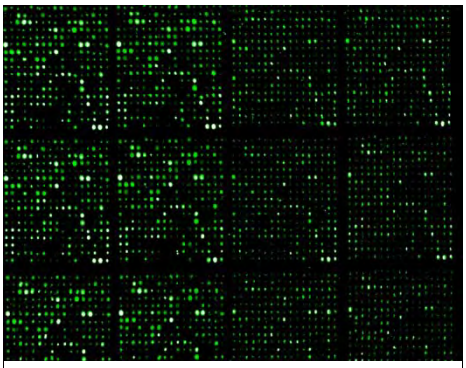

Figure 1 NYU Glycochip Developed with a Standard Serum. The two leftmost columns contain glycans in 50µM concentrations. The two leftmost columns contain glycans in 10 µM concentrations.

Data Management and Quality Control

The NYUSoM glycan array includes sixteen subgrids each of which contains 288 unique features including a positive control (biotin) and negative control

(printing buffer). Eight of the subgrids have a glycan concentration of 50µM. The remaining eight subgrids have a glycan concentration of 10µM (Figure 1). Because of the variable size of array spots it was determined that total spot intensity was the most accurate measure of signal intensity. Due to the large number of outliers in signal totals, medians of the eight replicates in each concentration were used to calculate intensities of spots for quality control. Custom software was developed to calculate medians of signal intensities using MATLAB (MathWorks). Detailed records of each quantified slide were kept and stored in a centralized, searchable, custom database. Records can be searched and sorted based on serum, slide barcode, date, experiment, external and internal conditions, print and print batch numbers.

Upon consultation with Marko Vuskovic ideal measurement for quality control of microarray developments was determined to be Lin's Concordance Coefficient, which measures the agreement between two variables. In our case the concordance coefficient measures the reproducibility of two slides. Based on previous data, a target concordance was set at .9 for any two given biological replicates. During the period in which we obtained slides from Scripps, an average concordance of approximately .7 was observed between biological replicates due to technical limitations. Consistency of slide quantification was achieved with an average concordance >.99 between operators of Imagene quantification software during this period. Custom software was created to calculate Lin's Concordance on large numbers of slide pairs from our database.

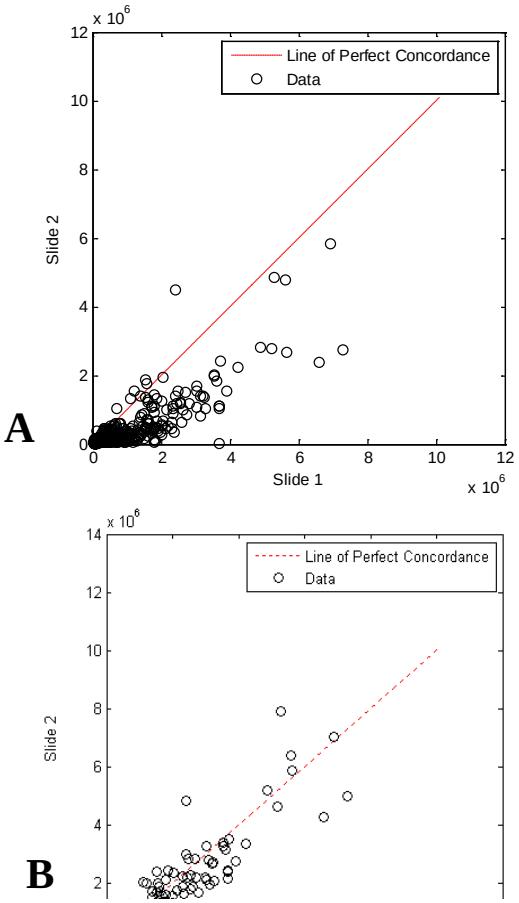

Figure 2- Concordance increases due to changes in protocol. A Slides with Low Lin Concordance- Median signal intensities of a sample slide 1 (X-axis) plotted against paired median signal intensities of slide 2(Y-axis). Dashed line represents perfect concordance. Lin's Concordance: .73 B Slides with High Concordance- Median signal intensities of a sample slide 1 (X-axis) plotted against paired median signal intensities of slide 2 (Y-axis). Dashed line represents perfect concordance. Lin's Concordance: .91

Initial Arraying Training and Printing

After the placeholder microarrayer was in working operation, technicians began to develop gridding protocols as well as environmental and mechanical calibrations mimicking the conditions printing occurred in Scripps. All basic environmental mechanical, environmental and technical issues were resolved in early December. At this time Ryan McBride, an arrayer at Scripps, trained the NYU technicians in glycan printing for a period of one and a half weeks. After Mr. McBride's visit, our staff was able to print a full microarray according to the protocol used at Scripps. Like the slides printed at Scripps, NYU slides were found to have low consistency between prints and developments. The next two months were spent minimizing inconsistencies in development and printing. Additional slide washes were added and concentrations of

our buffers used in slide development were changed. In order to minimize evaporation during printing stock microplates were loaded individually by hand, keeping them at a low temperature for a maximum period of time thereby minimizing evaporation. Additionally, the staff at Scripps had changed the concentration of printing buffer used, which was a large contributor to the inconsistencies in the data. After finding an optimal salt concentration of printing buffer in early February, the consistency and signal strength of data increased dramatically. Consistent data was briefly achieved in mid-February, followed by a period when data consistency dropped sharply. The source of this inconsistency was determined to be caused by the microarray scanner. Once the issue was fixed, target consistency was achieved.

Consistent Printing and Pilot Studies

In seven biological replicates belonging to different prints and developments, an average concordance >.90 was achieved with concordance of all pairs above .80 (Figure 2). The consistency of our developments was then tested using test samples as opposed the standard serum that had been used for optimization. Once consistency was confirmed, small pilot studies were undertaken to assure that slide production and development could scaled up. The first study focused on differences when the temperature and pH of hybridization were changed to conditions found in tumors (pH of 6.8, 39 ^{o}C). A very high degree of consistency between slides developed in the two conditions was found. In order to test on-site database software, analysis, and quality control without sacrificing our trial sera serum provided to us by Dr. Steven Albeda at the University of Pennsylvania was developed using NYU procedures. The study, performed in late April, early May, confirmed that the customized software was suitable for instant quality control of samples and that it was possible to scale up to trial volume.

DOD Meso Trial

At the current pace 16 samples will be developed per day as well as periodic quality control samples (standard serum) and repeats of low quality and failed developments. The development is slated to finish by August 2011 at which time the samples will be sent to Marko Vuskovic for processing and algorithm development. Additionally, the new Microgrid II unit was delivered and made operational in early May. Slides from the new unit had high concordance with slides from the older unit.

Animal Studies

We have received approval to start our animal studies in which asbestos and control fibers are delivered to the animals both from NYU as well as the DOD. We fully expect this to start in June 2011. The secondary animal protocol in which we will be using rat xenografts has been delayed by mycoplasm infection in the laboratory, and we are presently treating these cells so that we may re submit the protocol with clean serologies for the cell lines. We anticipate this starting also in June 2011.

KEY RESEARCH ACCOMPLISHMENTS

1. We have constructed the new glycol laboratory and validated the consistency of the slides that will be used for all these studies.
2. We have all the protocols approved for the first animal study for the asbestos model of mesothelioma in rats.

REPORTABLE OUTCOMES

None

CONCLUSIONS

It is now up to us to revise our plans in order to accomplish the SOW in a narrower window. We do not feel that this is a problem at this time, and we appreciate the support for this ongoing effort.